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Chromatin accessibility profiling of Treg cells in acute urticaria

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ABSTRACT

Acute urticaria can be a presenting symptom of anaphylaxis characterized by transient red swellings or fulminant wheals, often accompanied by severe pruritus. Numerous studies have substantiated the important involvement of regulatory T cells (Tregs) in the occurrence of allergic diseases and autoimmune diseases. However, the role of Tregs in the pathogenesis of acute urticaria is unclear. In this study, we found that the frequency of Tregs in peripheral blood mononuclear cells (PBMCs) was decreased in patients with acute urticaria compared with normal controls by flow cytometry. Analysis of Assay for transposase-accessible chromatin with sequencing (ATAC-seq) data identified 28 differentially accessible regions comparing Tregs from healthy individuals and patients with acute urticaria, all showing increased chromatin accessibility in the Tregs from acute urticaria. IL-1b was highly expressed in sera of patients with acute urticaria and the level of IL-1b was moderately positively related to white blood cell count. The elevated expression of IL-1b may be due to the diminished immune-suppressive function following the decline of Tregs in this study. We found that *IL1B* gene expression was also significantly increased in the skin lesions of both chronic spontaneous urticaria and solar urticaria compared to healthy controls. IL1B might play a key role in the development of acute urticaria and IL1B could be a potential prognostic biomarker and therapeutic target in urticaria.

KEY POLICY HIGHLIGHTS

- In patients with acute urticaria, the proportion of special immune cells called regulatory T cells in their blood was lower compared to healthy individuals.
- A special test called ATAC-seq found 28 areas in the DNA of regulatory T cells that are more easily accessed in patient with acute urticaria than in healthy individuals.
- A molecule called IL1B was found to be increased in the blood and skin of patients with different forms of urticaria and might be involved in the condition.

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Acute urticaria; chronic urticaria; regulatory T cells; the assay for transposase-accessible chromatin with sequencing (ATAC-seq); chromatin accessibility; IL1B

Introduction

Urticaria is a common, mast cell-driven heterogeneous inflammatory dermatosis characterized by sudden and unpredictable pruritic wheals with/without angioedema. It can be classified into acute and chronic urticaria based on symptom duration. Acute urticaria may develop spontaneously, or as a presenting feature of anaphylaxis lasting no more than 6 weeks. Chronic urticaria refers to symptom duration more than 6 weeks, which can come with daily or almost daily signs, or recur intermittently following variable periods of remission [1]. Immediate type I hypersensitivity reaction to multiple exo-allergens such as infection, medicines, foods, microorganisms, pollen or other factors is identified in ~ 50% acute urticaria cases [2]. Among them, infection has been identified as a frequent cause of acute urticaria in children [3]. These exo-allergens bind to immunoglobulin E (IgE)-FcεRI complex on mast cells and basophils resulting in their degranulation and subsequent symptoms [4]. Additionally, it has been discovered that angiotensin-converting enzyme (ACE) inhibitor can induce urticaria by elevating bradykinin levels. In patients with urticaria caused by nonsteroidal anti-inflammatory

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drugs (NSAIDs), the arachidonic acid pathway is implicated, possibly with an inhibition of prostaglandin synthesis and an increase in leukotrienes. These mechanisms are independent of IgE-mediated pathogenesis [3].

Regulatory T cells (Tregs) are a heterogeneous group of T-cell subsets with suppressive capacity that play critical roles in maintaining peripheral immune tolerance and preventing the development of both allergic and autoimmune diseases [5,6]. At present, it is generally accepted that the production and maintenance of allergen-specific Tregs and their involvement in inhibiting cytokines and surface molecules are essential for inducing allergen tolerance [7].

Recent studies have suggested that Tregs play an important role in the pathogenesis of urticaria [8–10]. For example, Arshi et al. found that the percentage of Tregs in peripheral blood mononuclear cells (PBMCs) decreased in patients with chronic urticaria, compared to control subjects. Yang et al. revealed a correlation between Th17/Treg immune dysregulation and the severity of chronic urticaria in patients. Among them, Tregs exhibited a significant negative correlation with pruritus level, disease severity, and impaired quality of life in chronic urticaria [11]. Conversely, another study found that Tregs increased and exhibited defective functions in PBMCs of patients with chronic urticaria [12]. However, the proportion of Tregs in acute urticaria and the role of Tregs in the pathogenesis of acute urticaria have not been studied.

The assay for transposase-accessible chromatin with sequencing (ATAC-seq) is a recent technology that identifies novel enhancers, gene regulatory regions or transcription factor activity and detects the unique chromatin accessibility landscape at base pair resolution for a given cell type or cell context of interest [13]. Differential activity of regulatory elements has been found in a wide range of diseases, such as primary human cancers [14], systemic sclerosis [15], Alzheimer's disease [16], and these findings motivate efforts to use ATAC-seq to characterize chromatin landscapes in large patient cohorts. This technology provides an opportunity to study the regulatory mechanism of acute urticaria and further understand the transcriptional regulatory programmes of acute urticaria gene regulatory networks.

In this study, we aimed to study genome-wide analysis of open chromatin in Tregs for acute urticaria and normal controls by ATAC-seq, and obtain the differences in chromatin accessibility patterns through joint analysis, which provided more clues for further research on the role of Treg cells in acute urticaria.

Materials and methods

Participants and experimental samples

We collected 3 batches of blood samples from male and female patients with acute urticaria who were hospitalized in the Department of Dermatology and Venereology of the First Affiliated Hospital of Anhui Medical University. The first batch of samples for flow analysis included 6 patients with acute urticaria (mean age 41.17 ± 21.05 years) and 6 healthy controls (mean age 33.50 ± 5.54 years). The second batch of samples for the ATAC-seq included 9 patients with acute urticaria (mean age 33.44 ± 16.09) and 6 healthy controls (mean age 30.33 ± 7.20). The third batch of samples for cytokine detection included 11 patients with acute urticaria (mean age 37.82 ± 14.49) and 7 healthy controls (mean age 29.00 ± 10.00). All patients met the diagnostic criteria for acute urticaria, excluded allergies, asthma, allergic rhinitis and other diseases such as tumors, autoimmune diseases etc. or pregnancy, and had not undergone regular glucocorticoid, biologics, allergen immunotherapy and anti-inflammatory drugs before enrolment. All controls were recruited without acute urticaria, allergies, asthma, or any other autoimmune disease. All enrolments were approved by the bioethics committee of the First Affiliated Hospital of Anhui Medical University and signed a written informed consent. The clinical information table of the enrolled patients is shown in Table 1. Peripheral venous blood collected (5–10 ml) from each patient and healthy control was used to extract PBMC or serum according to the standard centrifugation method.

Main reagents

TruePrep™ DNA Library Prep Kit V2 for Illumina® (Vazyme TD502), TruePrep™M Index Kit V2 for Illumina® (Vazyme TD202), VAHTS DNA Clean

Table 1. Clinical laboratory parameters of all acute urticaria subjects.

Batch of samples	Case No	Age, years	Sex	CRP (0–10.00 mg/L)	WBC (3.50–9.50 × 10^9/L)	IL-1b (0–5.00 pg/mL)	Clinical symptom
Flow cytometry analysis	1	68	Female	6.62	3.14	NA	rash
	2	19	Female	24.50	8.60	NA	rash
	3	53	Female	7.16	7.58	NA	rash
	4	13	Male	5.93	9.76	NA	rash
	5	48	Female	30.18	19.64	NA	rash, chest tightness
	6	46	Female	0.37	14.67	NA	rash
ATAC-seq study	1	24	Female	0.90	4.33	NA	rash, chest tightness
	2	30	Female	1.36	12.25	NA	rash, chest tightness, abdominal pain
	3	18	Male	55.90	20.01	NA	rash, laryngeal obstruction, abdominal pain
	4	19	Male	30.17	15.87	NA	rash, abdominal pain
	5	25	Male	29.87	11.68	NA	rash, chest tightness
	6	22	Female	38.10	21.97	NA	rash, chest tightness
	7	57	Female	0.50	16.23	NA	rash, laryngeal obstruction, abdominal pain
	8	53	Female	11.40	7.91	NA	rash, chest tightness
	9	53	Female	7.14	19.55	NA	rash, chest tightness, abdominal pain
Cytokine detection	1	60	Female	31.80	12.28	8.05	rash, abdominal pain
	2	22	Female	30.29	20.92	9.86	rash, abdominal pain
	3	23	Female	31.07	18.37	16.40	rash, abdominal pain
	4	33	Male	19.07	18.89	17.90	rash, chest tightness, abdominal pain, diarrhea
	5	32	Male	84.11	18.36	7.92	rash, abdominal pain
	6	60	Female	6.16	8.95	8.75	rash, abdominal pain and diarrhea
	7	32	Female	98.80	25.19	26.20	rash
	8	54	Male	1.56	12.94	6.05	rash, chest tightness
	9	38	Female	6.06	16.70	6.80	rash, chest tightness
	10	41	Female	12.45	6.86	8.58	rash
	11	21	Male	38.50	10.26	9.67	rash

CRP, C-reactive protein; WBC, white blood cell; NA, This examination was not performed.
The values in parentheses are the reference ranges.

Beads (Vazyme N411) were purchased from Vazyme Biotech Co.,Ltd (China). CD4 MicroBeads, human (120000440), CD25 MicroBeads II, human (130092983), OctoMACS™ Separator, quadroMACS™ Separator, LS, and MS separation columns were purchased from the German Company, Miltenyi Biotec. BV421 Mouse Anti-Human CD3 (562426), FITC Mouse Anti-Human CD4 (550628), PE-Cy™7 Mouse Anti-Human CD127 (560822), APC Mouse Anti-Human CD25 (555434) were purchased from the US Company, BD Pharmingen™. Immulite/immulite®1000 IL1B (Siemens) was purchased from UK.

Isolation of CD4+CD25+CD127- Tregs from PBMC

CD4 MicroBeads, human (20ul/10^7) were added to PBMC and incubated for 30 minutes at 4°C in a refrigerator, 1 ml buffer was added to resuspend the cell suspension, the LS column was put into quadroMACS™ Separator device, the sorting column was rinsed with 1 ml buffer for 2 times, and the cell suspension was added to the sorting column. The column was rinsed with buffer three times to collect the suspension. After centrifuging, the supernatant was discarded and 90 ml buffer added to resuspend before adding CD25 MicroBeads II, human (20ul/10^7) to the cell suspension. After incubating at 4°C in the refrigerator for 30 minutes, 1 ml buffer was added to resuspend the cell suspension, the MS column was put into the OctoMACSTM Separator device, and the sorting column washed twice with 500ul buffer. The cell suspension was added to the column, the column was rinsed 3 times with buffer, and the suspension was collected by adding 1 ml buffer in the MS column and pushing it down to the bottom of the column with a piston quickly.

Flow cytometry analysis of the proportion of CD4 +CD25+CD127- Tregs

We added BV421 Mouse Anti-Human CD3, FITC Mouse Anti-Human CD4, PE-CyTM7 Mouse Anti-Human CD127, APC Mouse Anti-

Human CD25 to the human single-cell suspension, incubated it under 4 degrees for 30 min, centrifuged it at 1500 r/min for 5 min, discarded the supernatant, washed it with buffer, resuspended it with 200ul buffer, then collected data from CytoFlex instrument and analysed the proportion of Tregs on FlowJo V10.8.1 software. The data was subjected to statistical analysis using the Mann-Whitney U Test to compare acute urticaria with the control group. A P-value threshold of less than 0.05 was used to determine statistical significance. Graphical representations were generated using GraphPad Prism software, version 10.

ATAC-seq

To study the landscape of chromatin accessibility in Tregs from acute urticaria, we generated ATAC-seq profiles from Tregs. ATAC-seq library was constructed for the second batch of 9 cases and 6 healthy controls. Tregs were extracted to prepare cell nuclear suspensions with cell viability greater than 90%. After splitting the nucleus, Tn5 transposase was added to break the DNA, and then incubated in a PCR machine at 37 degrees for 30 minutes. The Tn5-mediated DNA fragment were selected with VAHTS DNA Clean Beads on a magnetic rack, and the selected DNA fragment was subjected to PCR amplified reaction, then the collection was carried out again with VAHTS DNA Clean Beads on a magnetic rack. The concentration of the DNA library was detected by Qubit 3.0 Concentration Meter, and the distribution of library fragments was detected by Agilent 2100. Finally, the ATAC library was submitted to the Wuhan Bioresource Sample Bank, and 10 × genomics sequencing was performed on the Illumina Novaseq 6000 sequencing platform (10 × Genomics, USA), generating an average of 12 G of data volume.

Bioinformatic analysis

The Fasta file for the human reference genome GRCh38 was downloaded from UCSC (https://genome.ucsc.edu). Quality control of ATAC-seq sequencing data was performed by aligning reads to the reference genome using Bowtie2; ATAC-seq peaks were determined using MACS2. Principal Component Analysis (PCA) was performed for all peaks from both patients and normal controls using Bioconductor package DiffBind, which was primarily developed for differential binding analysis of ChIP-Seq peak data. DiffBind was also used to identify differentially accessible peaks associated with acute urticaria compared with the control group. By default, the peaks in the consensus peakset were re-centered and trimmed based on calculating their summits in order to provide standardized peak intervals. The binding affinity matrix containing a read count for all samples was next fed to DESeq2. Differential binding affinity analysis was performed using the default unpaired design. ChIPseeker was used to annotate peaks overlapping known transcription start sites (TSSs), promoters, exons, and introns. Enrichment Analysis (GSEA) Application of GSEA (v.4.1.0) was used to do enrichment analysis for genes related to the differentially accessible peaks. The significant threshold was set as $p < 0.05$.

Cytokine detection

Serum samples were collected from 11 patients with acute urticaria and 7 healthy controls to measure the level of circulating cytokine IL-1b using the technique of a solid-phase, two-site chemoluminescent enzyme immune-metric assay (Immulite 1000, Siemens, UK). Differential expression level for IL-1b was performed using two-sample t test. The relationship between IL-1b levels and WBC count in acute urticaria was indicated by Pearson correlation coefficient.

Transcriptome data mining

To explore the expression levels of target genes by ATAC-seq in other types of urticaria, we retrieved genome-wide gene expression datasets for chronic spontaneous urticaria (CSU) from public GEO databases (GSE72542) for differential expression analysis. The transcriptome data included 20 patients suffering from severely active CSU and 10 healthy controls. Meanwhile, we also searched the ArrayExpress database (accession numbers E-MTAB-12218

[HC] and E-MTAB-12219 [solar urticaria]). The transcriptome data included 4 patients with solar urticaria and 4 healthy controls. R package 'ggpubr' was used to visualise the expression of target genes in normal control and case. Differential expression levels for target genes were performed using two-sample t test, and the *p* value was corrected by Benjamini and Hochberg FDR (BH) method.

Results

The clinical characteristics of all acute urticaria subjects

All patients were experiencing a rash, with some also reporting symptoms of chest tightness and/or abdominal pain. Most patients were likely induced by infection, some from food and environmental factors, and a few were idiopathic with no identified cause. White blood cell (WBC) count, percentage of neutrophils and C-reactive protein were abnormally elevated in most acute urticaria cases (Table 1). Besides, D-Dimer and fibrin degradation products also increased significantly in some cases. Percentage of lymphocytes, hemoglobin and platelets were not significantly abnormal (Supplementary Table S1).

The frequency of Tregs in patients with acute urticaria and controls

The frequency of CD4+CD25+CD127- T cells within peripheral CD4+ T cells population is one approach for characterizing populations of Tregs [17]. We observed a reduced proportion of CD4 +CD25+CD127- Tregs in acute urticaria patients when compared with healthy controls. The median proportion of Tregs in PBMCs observed was 2.2% in acute urticaria patients versus 5.1% in controls ($p < 0.05$) (Figure 1).

Chromatin accessibility landscapes of Tregs in acute urticaria

The ATAC-seq libraries were sequenced to obtain an average of more than 3 million paired-end reads per sample. From these data, a total of 84,004 high-quality peaks were identified in acute urticaria and control Tregs. The average percentage of reads in peaks (RiP%) was 5.35%. Principal component analysis (PCA) results showed the unsupervised clustering of distinct chromatin signatures of Tregs in cases and controls (Figure 2(a)), suggesting further chromatin remodelling in the pathogenesis of disease. Functional state annotation from ChIPseeker revealed that chromatin remodelling in Tregs occurred mostly at distal intergenic and other intron regions (Figure 2(b,c)), and the positional distribution of transcription factor-binding loci was enriched in the region both from 10 kb to 100 kb upstream and downstream of the transcription start site (TSS).

ATAC-seq reveals regulatory signatures of Tregs in acute urticaria

To better understand the regulatory genome of Tregs in acute urticaria, the differential analysis of chromatin accessibility signal within these peak regions was performed between acute urticaria and control samples using established pipelines, and serve as the starting point for a variety of downstream analyses. ATAC-seq identified 28 regions which were differentially accessible between healthy individuals and patients with acute urticaria, and all of which displayed increased chromatin accessibility in the Tregs from acute urticaria (Table 2). The top differentially accessible peaks were located around *SPATA13*, *TPRG1* and *IL1B* genes. Peak functionality can partially be inferred by annotating peaks to the nearest gene. We depicted the genome tracks of the *IL1B*, *SPATA13*, *TPRG1* and *IPCEF1* loci as four examples showing the regions that were differentially more accessible in the Tregs of acute urticaria compared to controls, as indicated by the increase in peak intensity (Figure 3, Supplementary Figures S1-S3).

To gain insight into the functions of the genes corresponding to differential peaks obtained by ATAC-seq in the Tregs of acute urticaria, we performed functional enrichment analyses by Gene Set Enrichment Analysis (GSEA) and found these genes were highly enriched for protein modification process, Foxp3 activated CD4+ T cell process,

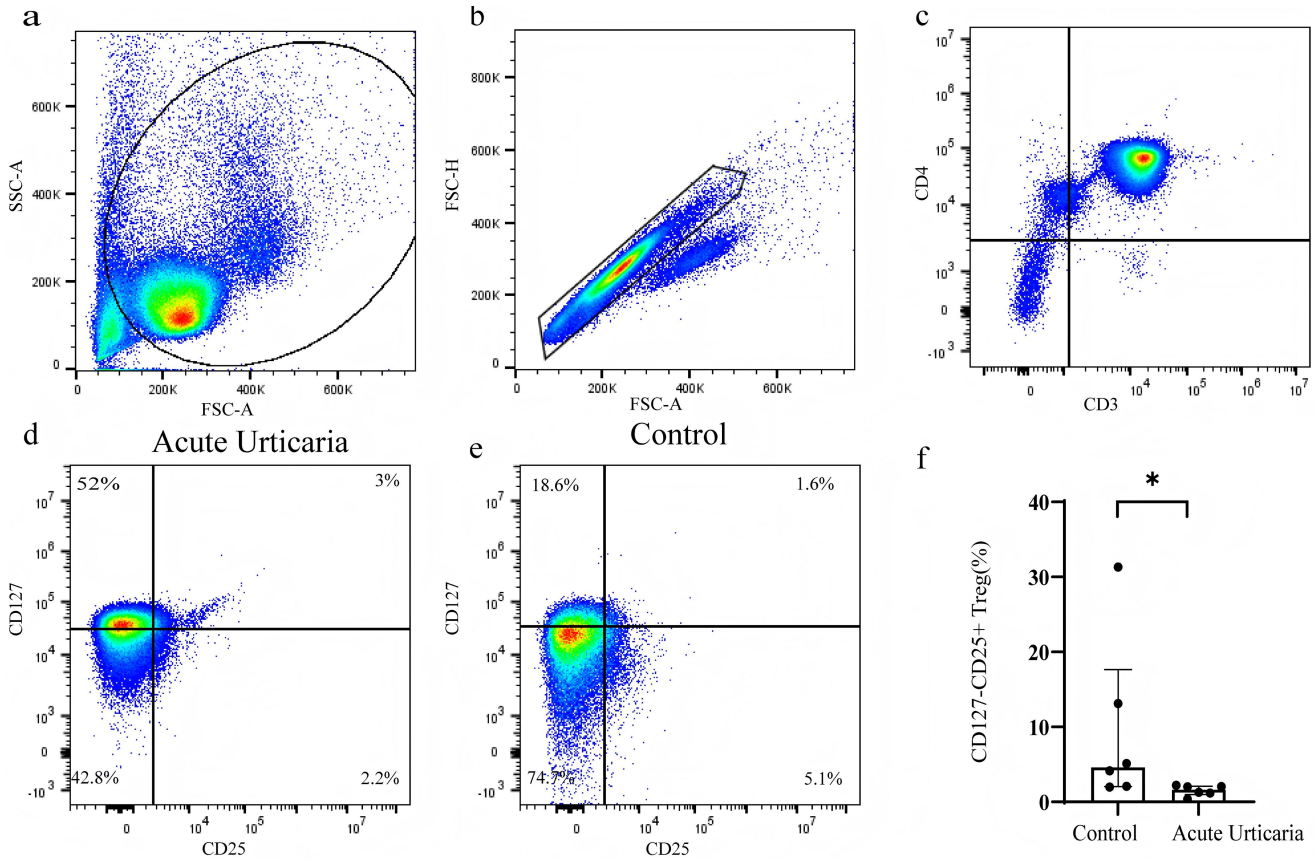

Figure 1. Flow sorting and detection of regulatory T cells in acute urticaria.

(a) The proportions of CD4+CD25+CD127- regulatory T cells in acute urticaria patients and healthy controls were analyzed. Lymphocytes were initially gated based on their relative side scatter (SSC-A) and forward scatter (FSC-A). (b) Subsequently, lymphocytes were further refined using side scatter height (SSC-H) and forward scatter area (FSC-A) to exclude debris and unwanted cells. (c) Within this population, CD3+CD4+ cells were identified and gated as regulatory T cells. (d)(e) Further gating was performed to specifically isolate CD4+CD25+CD127- regulatory T cells. (f) Statistical comparisons between the two groups were visualized in the corresponding figure. *$p < 0.05$.

macromolecule metabolic process and Tconv vs Treg precusors process (Figure 4).

Subsequently, we measured the expression level of cytokine IL-1b in 11 acute urticaria patients and 7 controls. IL-1b was significantly elevated in sera of patients with acute urticaria compared to controls ($p = 0.0061$, Figure 5(a)). Moreover, we also found that the expression level of IL-1b was moderately positively correlated with WBC count (correlation coefficient = 0.67, $p = 0.024$, Figure 5(b)).

To understand the expression levels of target genes by ATAC-seq and identify the differentially expressed genes in skin tissue, we extracted the public transcriptome data of CSU [18] and solar urticaria [19]. We separately calculated the expression patterns of 24 of these 28 target genes corresponding to differential peaks obtained by our ATAC-seq between patient and control groups. In transcriptome data obtained from lesional and nonlesional CSU (LS and LN, respectively) and healthy control skin, *CCNH*, *IL1B*, *NETO2*, *TPRG1* were up-regulated genes and *DIAPH3*, *NR3C1*, *PPM1A*, *SMARCA2* were down-regulated genes in lesional skin when compared with control skin (Figure 6(a,b)). In the solar urticaria transcriptome data, *CCNH*, *GLB1*, *IL1B*, *TPRG1* were up-regulated genes and *NR3C1*, *SMARCA2*, *ZNF536* were down-regulated genes in lesional skin (Supplementary Figure S4(a-b)). The figures showed that *IL1B* gene expression was significantly increased in the skin lesions of both CSU and solar urticaria compared to control skin.

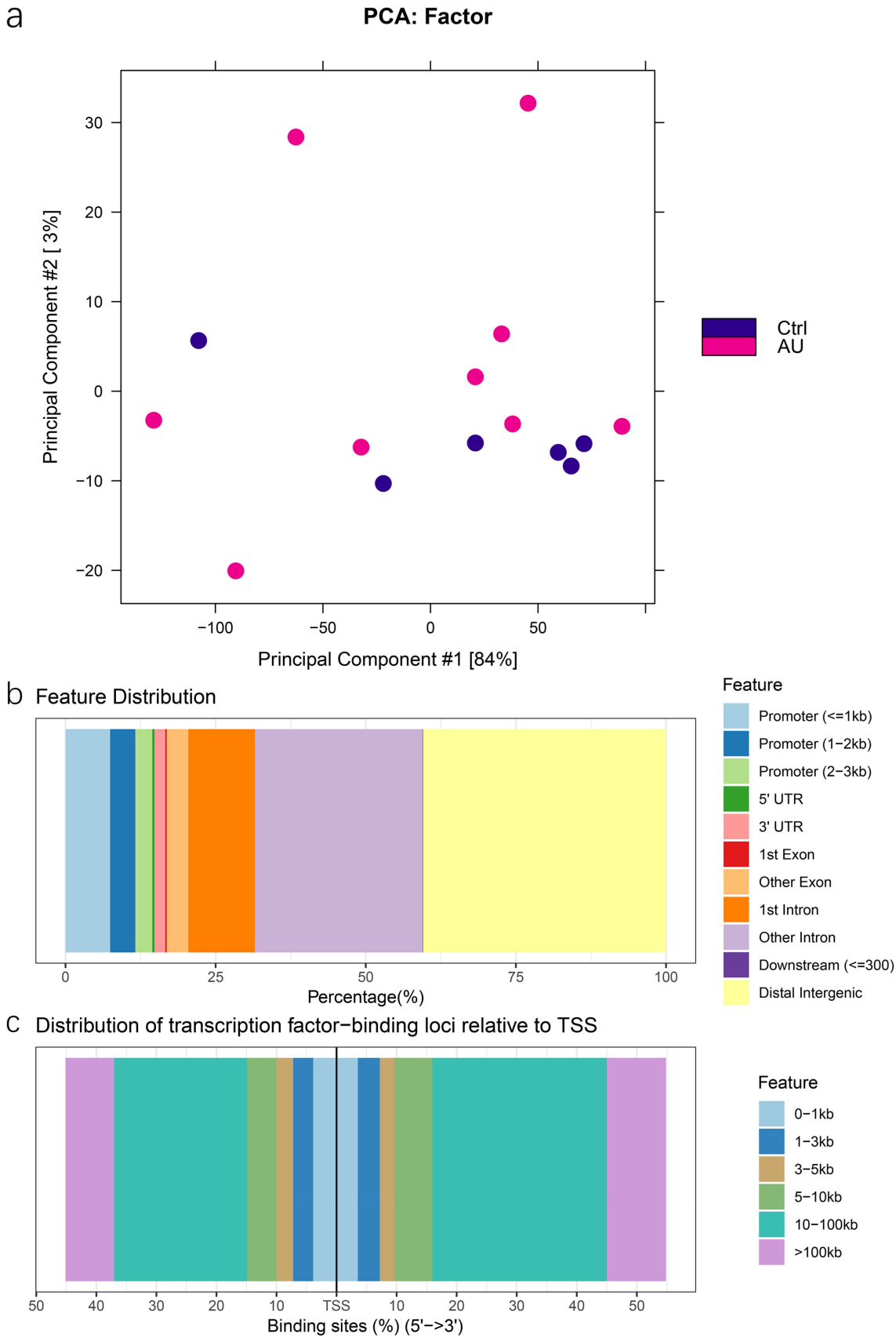

Figure 2. Chromatin accessibility landscapes of Tregs in acute urticaria.

(a) Principal component analysis of Tregs in nine patients with acute urticaria and six normal controls. AU: acute urticaria; Ctrl: controls. (b) Major functional state annotation of peaks in Tregs. (c) The positional distribution of transcription factor–binding loci relative to TSS in Tregs.

Table 2. The differential analysis results of peaks in the Tregs from acute urticaria patients and controls.

seqnames	start	end	Conc_Case	Conc_Control	p.value	annotation	SYMBOL
chr13	24683865	24684265	3.75	2.14	0.0061	Intron	SPATA13
chr3	188788490	188788890	4.36	2.45	0.0064	Intron	TPRG1
chr6	141483651	141484051	6.55	4.36	0.0112	Distal Intergenic	MIR4465
chr2	113626915	113627315	3.58	1.87	0.0161	Distal Intergenic	IL1B
chr14	60753800	60754200	4.86	3.09	0.0167	3′ UTR	PPM1A
chr6	154675598	154675998	5.21	3.55	0.0187	Promoter (1-2kb)	IPCEF1
chr2	102227640	102228040	4.25	3.09	0.0219	Distal Intergenic	MAP4K4
chr13	59391646	59392046	5.22	3.29	0.0227	Distal Intergenic	DIAPH3
chrX	20412831	20413231	4.63	3.00	0.0234	Distal Intergenic	RPS6KA3
chr5	102780432	102780832	3.67	2.11	0.0250	Distal Intergenic	NUDT12
chr9	2053885	2054285	4.99	3.31	0.0278	Intron	SMARCA2
chr5	87081479	87081879	3.50	1.71	0.0291	Distal Intergenic	CCNH
chr5	142713072	142713472	4.45	3.19	0.0302	Intron	NR3C1
chr14	97709568	97709968	3.70	2.41	0.0346	Distal Intergenic	LINC02291
chr16	82655383	82655783	4.80	3.26	0.0353	Distal Intergenic	CDH13
chr16	19523656	19524056	4.97	3.70	0.0376	Intron	GDE1
chr3	8357205	8357605	6.15	4.36	0.0399	Intron	LMCD1-AS1
chr6	141848526	141848926	4.52	2.83	0.0404	Distal Intergenic	NMBR
chr3	141498760	141499160	5.70	4.21	0.0404	Promoter (1-2kb)	GRK7
chr2	178414624	178415024	4.50	3.38	0.0408	Promoter (2-3kb)	TTC30B
chr3	67655687	67656087	5.32	3.99	0.0414	Intron	SUCLG2
chr3	59451346	59451746	6.03	4.35	0.0419	Distal Intergenic	CFAP20DC
chr10	12448806	12449206	5.16	3.77	0.0432	Intron	CAMK1D
chr3	33096966	33097366	4.49	3.49	0.0444	Intron	GLB1
chr5	131382345	131382745	4.87	3.51	0.0450	Distal Intergenic	IL3
chr8	58801405	58801805	5.90	4.27	0.0465	Distal Intergenic	FAM110B
chr19	31214124	31214524	5.23	3.87	0.0488	Distal Intergenic	ZNF536
chr16	47141701	47142101	4.75	3.40	0.0499	Promoter (1-2kb)	NETO2

Conc_Case, the mean read concentration in acute urticaria samples; Conc_Control, the mean read concentration in healthy controls.

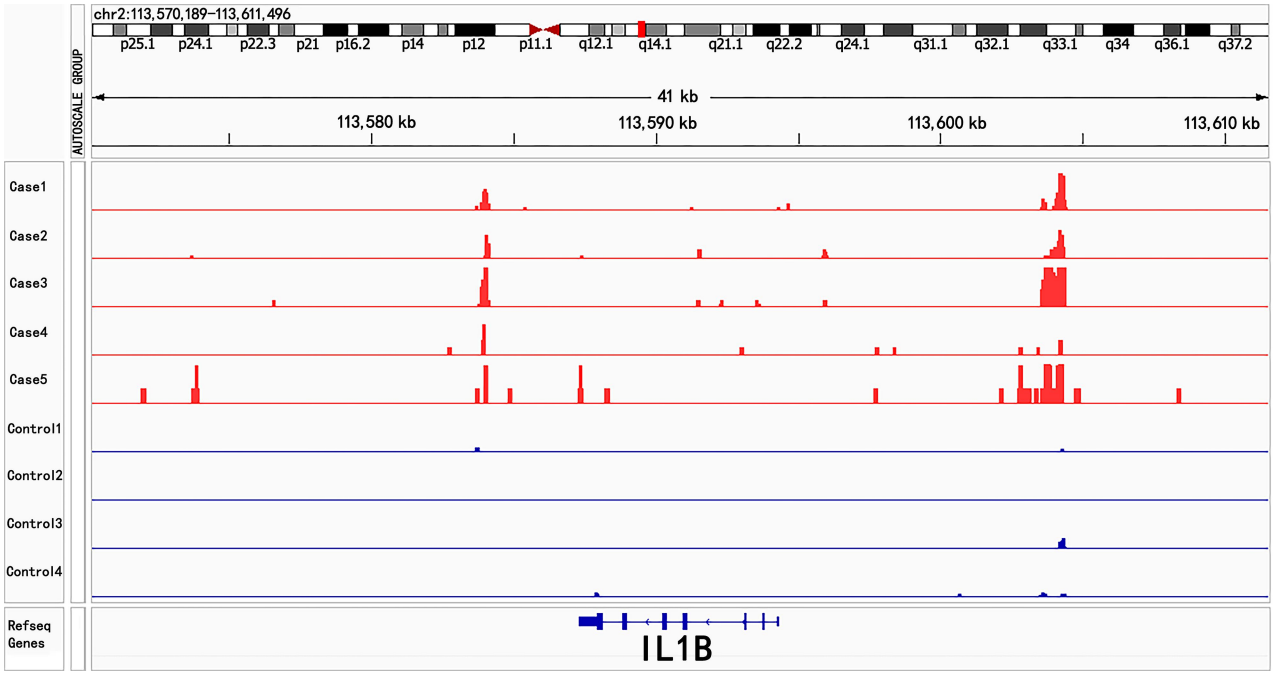

Figure 3. Representative ATAC-seq profiles at genes *IL1B* with differential peaks.

Discussion

Tregs are key mediators of peripheral tolerance and prevent autoimmune disease by modulating the immune responses, which may be the result of reduced Tregs numbers or altered function. Tregs target many different immune cell subsets and

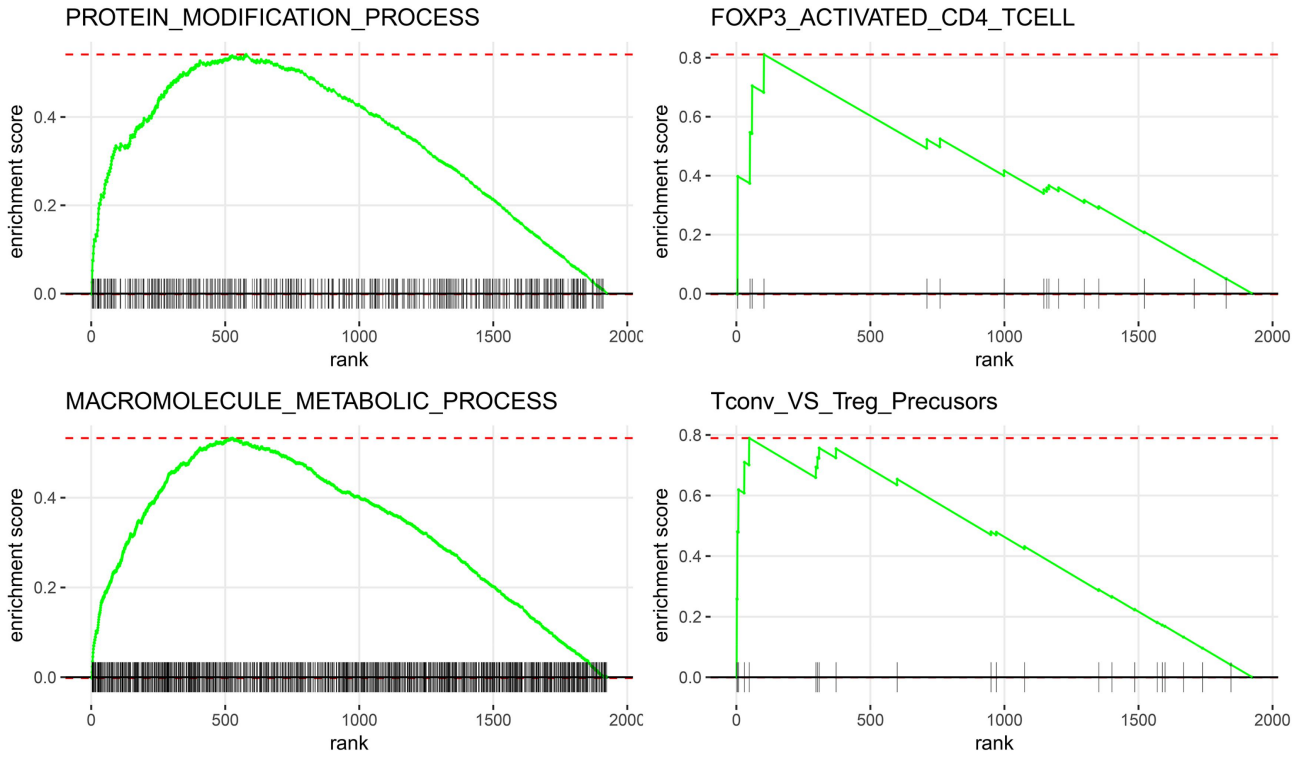

Figure 4. Pathway enrichment analysis of genes annotated to all differentially accessible regions in Tregs.

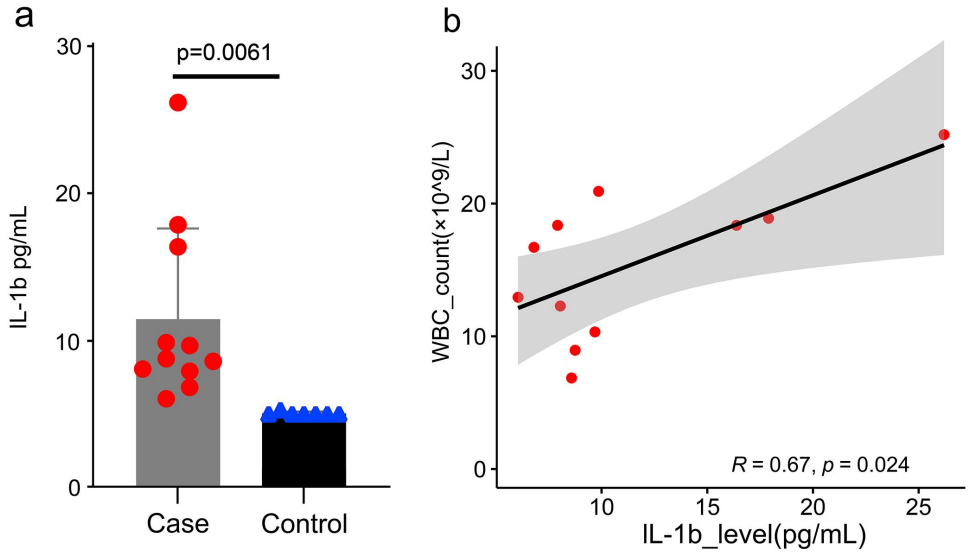

Figure 5. Serum levels of IL-1b in acute urticaria patients and controls and correlation with WBC count.
(a) This plot illustrates the levels of IL-1b in acute urticaria cases and controls. IL-1b was significantly elevated in sera of the case group. (b) This scatter plot illustrates the relationship between IL-1b levels and WBC count in acute urticaria. The correlation coefficient of 0.67 suggests a moderate positive relationship.

tissues to suppress excessive inflammation and maintain immune homeostasis and provide immune tolerance to both self and non-self-innocuous antigens. Tregs are also key players for reducing the inflammation-mediated tissue damage following infection. There are numerous surface receptors that are specific for defined Treg cell subsets, indicating the heterogeneity of this

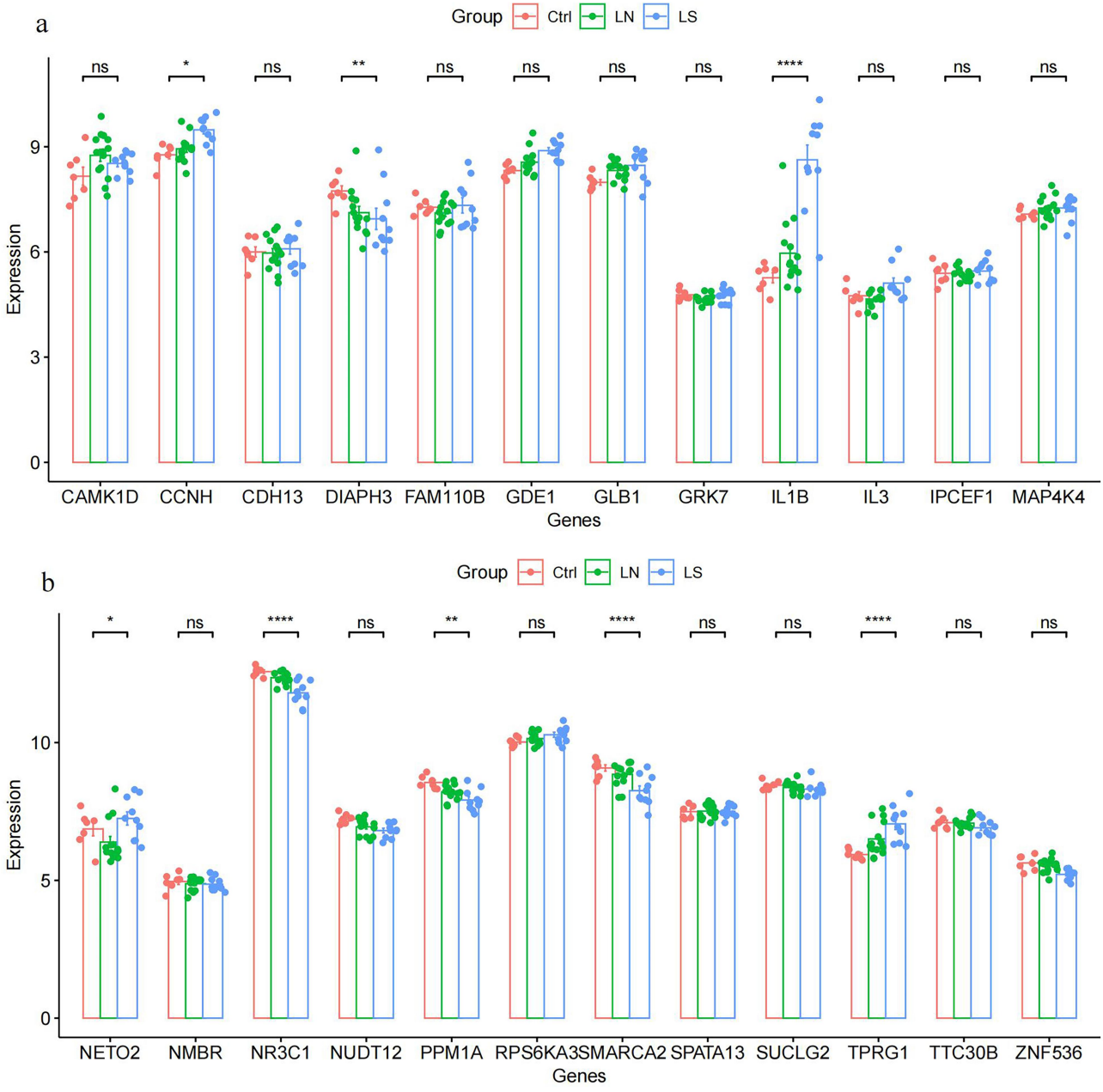

Figure 6. Differential expression analysis of the target genes in the transcriptome data of CSU.
(a)(b) The expression levels of 24 of the 28 genes, which correspond to the differential peaks from our ATAC-seq, were analyzed in the CSU transcriptome data. LS: lesional skin (wheal) from CSU; LN: non-lesional skin from CSU.

cell population [20]. Several studies have found that peripheral Treg deficiency is associated with several autoimmune skin diseases such as chronic urticaria, vitiligo, alopecia areata and systemic sclerosis [21–23].

The central role of IgE in allergic disease, especially in urticaria, is well recognized [6,24], and it is becoming increasingly clear that IgE also has a wider role in other diseases including autoimmune disease [25]. A study indicates that an oral immunotherapy protocol combined with the anti-IgE therapy omalizumab reduces the proliferation of allergen-reactive T cells during allergen desensitization, followed by a gain in allergen-specific Tregs function due to the suppression of their Th2 cell-like program with decreased IL-4 production. The functional restoration of Tregs may play a critical role in mitigating food allergy symptoms through oral immunotherapy facilitated by omalizumab [26]. Omalizumab has

been shown to restore Treg cell homeostasis in children with severe asthma, which is associated with clinical improvement and asthma control [27]. Recent finding also demonstrated that omalizumab restores the ability of human plasmacytoid dendritic cells to induce Tregs in vitro, suggesting a possible role for anti-IgE therapy in the management of allergic or autoimmune diseases that might be mediated by Tregs [28]. Future studies are needed to investigate the role of Tregs on the pathogenesis of urticaria.

In this present study, we firstly observed the proportion of Tregs in acute urticaria, and then performed ATAC-seq to examine chromatin accessibility in Tregs from patients with acute urticaria compared with normal controls, demonstrating significant alterations in chromatin accessibility.

Our study found that the frequency of Tregs in PBMCs was decreased in patients with acute urticaria compared with normal controls, which is consistent with previously reported results in chronic urticaria [8]. Although there are significant differences in the pathogenesis of acute and chronic urticaria, IgE mediation and mast cells activation are commonly implicated in the occurrence and progression of the two urticaria subgroups, with the exception of certain cases that are mediated through non-IgE pathways [24]. Foxp3, a specific marker for Tregs, is required for the development, function and stability of Tregs. Animal experiments show that serum levels of immunoglobulins including IgE are increased in Foxp3 knockout mice [6,29]. The observation that Foxp3 knockout mice exhibit several pathologic features associated with human allergic disorders, including spontaneous allergic airway inflammation, abnormally increased IgE level, and atopic dermatitis, suggests that Tregs deficiency and function abnormality are involved in the pathogenesis of allergic disorders. Such abnormalities might be related to failure to produce, develop, or expand Tregs, and/or failure to up-regulate suppression functions of Tregs once they enter the target organ or tissue. Foxp3 mutations also underlie a homologous autoimmune lymphoproliferative disorder in human subjects, termed immune dysregulation polyendocrinopathy enteropathy – X-linked syndrome (IPEX), which often exhibit skin symptoms, such as atopic dermatitis, psoriasiform lesions, idiopathic urticaria, alopecia areata and hyper-IgE [30,31]. Abnormalities in Tregs by Foxp3 deficiency might cause allergic dysregulation with IgE involvement, thereby contributing to the occurrence of various allergic diseases [32].

Thus, we performed the differential analysis of chromatin accessibility signal within these peak regions of Tregs between acute urticaria and control samples by ATAC-seq, and identified 28 regions with increased chromatin accessibility signal. Among them, *IL1B* loci showed differentially more accessible in the Tregs of acute urticaria compared to controls. *IL1B* gene encodes interleukin 1 beta, a member of the interleukin 1 cytokine family. This pro-inflammatory cytokine is an important mediator of the inflammatory response, and plays an essential role in cell proliferation, differentiation and apoptosis, but is also involved in several pathophysiological processes. IL-1b can be released from monocytes, macrophages, mast cell and dendritic cells leading to a series of inflammatory cascade responses like activation of immune cells and pyroptosis under the stimulation of immune response, inflammation and infection. Some studies have shown that Tregs can inhibit the production and modulate the effects of IL-1b, and IL-1b can also influence the function and activity of Tregs [33–36], which creates a regulatory loop that can either maintain immune tolerance or lead to immune dysregulation. In this study, the increased expression level of IL-1b may be due to the diminished immune-suppressive function following the decline of Tregs, leading to the activation and proliferation of other immune cells such as macrophages and dendritic cells [34,37]. Infection is one of the main pathogenic factors of acute urticaria. In this study, the majority of cases were likely due to bacterial infection, while a smaller number were associated with factors such as food, environmental influences. In some cases, no definitive cause was identified, suggesting that the condition may be idiopathic. Mast cells degranulated and produced IL-1b in response to bacterial infection through FcγRs and other inflammatory signals suggesting that IL-1b may be positively associated with mast cells activation [38,39].

Therefore, infection may be a significant factor in the elevation of IL-1b in this study. IL-1b can bind to the IL1 receptor (IL-1 R) on the surface of immune cell, leading to a cascade of intracellular signaling events. This interaction may result in the degranulation of mast cells, releasing histamine, proteases, and other mediators that contribute to inflammation and immune responses. IL-1b can also induce the production of additional cytokines and chemokines by mast cells, further amplifying the inflammatory response [40,41]. Nakamura et al. found that both IL-1b and mast cells are implicated in cryopyrin-associated periodic syndromes (CAPS), an autoinflammatory disease caused by aberrant IL-1b production and characterized by urticarial skin lesions [40]. In the light of the correlation between IL-1b and Tregs as well as mast cells, we measured the level of IL-1b and found that IL-1b was significantly elevated in sera of acute urticaria compared to controls. In addition, we investigated these target genes corresponding to differential peaks obtained by our ATAC-seq using the public transcriptome data of CSU and solar urticaria, and found that *IL1B* gene expression was also significantly increased in the skin lesions of both CSU and solar urticaria compared to control skin. These findings indicated that IL1B may participate in activation of mast cells and wheal formation during the onset of diseases and suggested that IL1B might play a key role in the development of acute and chronic urticaria and could be a potential prognostic biomarker and therapeutic target in urticaria.

Another region with increased chromatin accessibility involved *IPCEF1*. This has been reported to interact with ADP-ribosylation factor GTP exchange factors of the cytohesin family and function by modulating the cytohesin 2 activity [42]. IPCEF1 might be involved in IL-1b signaling and cell adhesion by binding cytohesin 2 [43,44], and was reported to be a risk loci with a function that is relevant to the pathophysiology of allergic disease [45]. *PPM1A* encodes the protein phosphatase, which is a member of the PP2C family of Ser/Thr protein phosphatases. Overexpression of this phosphatase is reported to activate the expression of the tumor suppressor gene TP53/p53. The role of PPM1A in regulating cellular signalling pathways is important for growth and stress response. PPM1A was a risk factor for allergic rhinitis in genome-wide association studies [46], and was also significantly elevated in allergic asthma, in which IgE, as an important mediator of allergic reactions, has a central role in pathophysiology [47]. Considered together, our findings suggested that PPM1A may be involved in the pathogenesis of acute urticaria. *NR3C1*, encoding glucocorticoid receptor, was reported to involve in glucocorticoid receptor signaling pathway, and could be a potential target in chronic urticaria and active hives [48].

CAMK1D encodes for a component of the calcium-regulated calmodulin-dependent protein kinase cascade and was identified as an epigenetic marker for allergic disease [49]. *GLB1* relevant with the lysosomal pathway was identified as differentially expressed gene in allergic asthma [50] and also was susceptibility loci for atopic dermatitis [51]. IL-3 is a potent growth promoting cytokine, which is secreted predominantly by activated mast cells, T-lymphocytes, basophils, eosinophils and osteoblastic cells. IL-3 has been identified among the most important cytokines for regulation of mast cells as well as basophils growth and differentiation, migration and effector function activities of many hematopoietic cells [52]. IL-3 increased the amount of histamine release by the sera from chronic urticaria which is able to activate basophils [53], and exhibited a higher expression level in the chronic urticaria group than in the control group [54]. A study suggested that IL-3 regulates FcεRI expression and cell survival in primary human basophils. Thus, blocking of IL-3 signaling in allergic effector cells might represent an interesting approach to diminish surface FcεRI levels and to prevent prolonged cell survival in allergic inflammation [55]. IL-3 May involve in severe hypersensitivity reactions and play a pivotal role in allergic disease, especially urticaria.

This study is subject to several limitations: First, the sample size was inadequate, which may limit the generalizability of our findings. Second, the experimental samples across the three phases were not drawn from the same batch due to disease-specific factors and time limitations. Additionally, most cases were likely induced by infection in this study, which may be a significant factor in the alterations observed in IL-1b and Tregs. No pre- and post-treatment

comparisons were conducted. The research was limited to Tregs, excluding other cell subtypes. Lastly, the potential relationship between IL1B and Tregs was not explored in depth. In the future, it is necessary to increase the sample size to improve statistical power and to further investigate the association between them.

Conclusions

In summary, we observed the frequency of Tregs in PBMCs was decreased in patients with acute urticaria and identified 28 regions with increased chromatin accessibility signal in the Tregs from acute urticaria by ATAC-seq. Among them, IL1B might play a key role in the development of acute urticaria and IL1B could be a potential prognostic biomarker and therapeutic target in urticaria. Further studies are needed to clarify the exact role of Tregs and differential chromatin accessibility signal in the pathogenesis of acute urticaria.

Abbreviations

Tregs	Regulatory T cells
PBMCs	Peripheral blood mononuclear cells
ATAC-seq	The assay for transposase-accessible chromatin with sequencing
IgE	Immunoglobulin E
WBC	White blood cell
PCA	Principal component analysis
GSEA	Gene Set Enrichment Analysis
Th	Helper T cells
CSU	Chronic spontaneous urticaria

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Authors' contributions

Conceived and designed the study: FSZ. Managed the project: LLW. Conducted experiments: LLW, XJZ. Data process and analysis: FSZ, QSY. Wrote manuscript: LLW, XJZ. All the authors revised and approved the manuscript to be published.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Availability of data and materials

The public transcriptome data of chronic spontaneous urticaria and solar urticaria used in this study is publicly available on the public GEO databases (GSE72542) and ArrayExpress database with the accession numbers E-MTAB-12218 [HC] and E-MTAB-12219 [solar urticaria], respectively. The original contributions during the study are included in this manuscript.

Consent for publication

All authors give consent for the publication of the manuscript

Ethics approval and consent to participate

This study was approved by the Institutional Ethical Committee of the Anhui Medical University and according to Declaration of Helsinki principles. Patient consent was also obtained. We are grateful to all study participants who donated blood samples for this study.

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References

[1] Zuberbier T, Abdul Latiff AH, Abuzakouk M, et al. The international EAACI/GA²LEN/EuroGuiDerm/APAAACI guideline for the definition, classification, diagnosis, and management of urticaria. Allergy. 2022 Mar;77(3):734–766. doi: 10.1111/all.15090

[2] Antia C, Baquerizo K, Korman A, et al. Urticaria: a comprehensive review: epidemiology, diagnosis, and work-up. J Am Acad Dermatol. 2018 Oct;79(4):599–614. doi: 10.1016/j.jaad.2018.01.020

[3] Sabroe RA. Acute urticaria. Immunol Allergy Clin North Am. 2014 Feb;34(1):11–21. doi: 10.1016/j.iac.2013.07.010

[4] Gimenez-Arnau AM, Manzanares N, Podder I. Recent updates in urticaria. Medicina Clínica. 2023 Nov 24;161(10):435–444. doi: 10.1016/j.medcli.2023.06.026

[5] Palomares O, Akdis M, Martin-Fontecha M, et al. Mechanisms of immune regulation in allergic diseases: the role of regulatory T and B cells. Immunol Rev. 2017 Jul;278(1):219–236. doi: 10.1111/imr.12555

[6] Palomares O, Elewaut D, Irving PM, et al. Regulatory T cells and immunoglobulin E: a new therapeutic link for autoimmunity? Allergy. 2022 Nov;77 (11):3293–3308. doi: 10.1111/all.15449

[7] Palomares O, Martin-Fontecha M, Lauener R, et al. Regulatory T cells and immune regulation of allergic diseases: roles of IL-10 and TGF-β. Genes Immun. 2014 Dec;15(8):511–520. doi: 10.1038/gene.2014.45

[8] Arshi S, Babaie D, Nabavi M, et al. Circulating level of CD4+ CD25+ FOXP3+ T cells in patients with chronic urticaria. Int J Dermatol. 2014 Dec;53(12):e561–6. doi: 10.1111/ijd.12630

[9] Sun RS, Sui JF, Chen XH, et al. Detection of CD4+ CD25+ FOXP3+ regulatory T cells in peripheral blood of patients with chronic autoimmune urticaria. Australas J Dermatol. 2011 Aug;52(3):e15–8. doi: 10.1111/j.1440-0960.2010.00658.x

[10] Mohamed A, Taha S, Ahmed A, et al. Correlation between regulatory T cells and autologous serum skin test among chronic spontaneous urticaria patients. Egypt J Immunol. 2022 Jul;29(3):99–106. doi: 10.55133/eji.290311

[11] Yang X, Chen L, Wang S, et al. The correlation between Th17/Treg immune dysregulation and the disease severity in chronic spontaneous urticaria patients. Immun Inflamm Dis. 2023 Jul;11(7):e920. doi: 10.1002/iid3.920

[12] Chen WC, Chiang BL, Liu HE, et al. Defective functions of circulating CD4+CD25+ and CD4+CD25– T cells in patients with chronic ordinary urticaria. J Dermatol Sci. 2008 Aug;51(2):121–130. doi: 10.1016/j.jdermsci.2008.02.012

[13] Grandi FC, Modi H, Kampman L, et al. Chromatin accessibility profiling by ATAC-seq. Nat Protoc. 2022 Jun;17(6):1518–1552. doi: 10.1038/s41596-022-00692-9

[14] Corces MR, Granja JM, Shams S, et al. The chromatin accessibility landscape of primary human cancers. Science. 2018 Oct 26;362(6413). doi: 10.1126/science.aav1898

[15] Liu Q, Zaba LC, Satpathy AT, et al. Chromatin accessibility landscapes of skin cells in systemic sclerosis nominate dendritic cells in disease pathogenesis. Nat Commun. 2020 Nov 17;11(1):5843. doi: 10.1038/s41467-020-19702-z

[16] Xu D, Zhang C, Bi X, et al. Mapping enhancer and chromatin accessibility landscapes charts the regulatory network of Alzheimer's disease. Comput Biol Med. 2024 Jan;168:107802. doi: 10.1016/j.compbiomed.2023.107802

[17] Seddiki N, Santner-Nanan B, Martinson J, et al. Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. J Exp Med. 2006 Jul 10;203(7):1693–1700. doi: 10.1084/jem.20060468

[18] Gimenez-Arnau A, Curto-Barredo L, Nonell L, et al. Transcriptome analysis of severely active chronic spontaneous urticaria shows an overall immunological skin involvement. Allergy. 2017 Nov;72(11):1778–1790. doi: 10.1111/all.13183

[19] Rutter KJ, Peake M, Hawkshaw NJ, et al. Solar urticaria involves rapid mast cell STAT3 activation and neutrophil recruitment, with FcεRI as an upstream regulator. J Allergy Clin Immunol. 2024 May;153(5):1369–1380. e15. doi: 10.1016/j.jaci.2023.12.021

[20] Dominguez-Villar M, Hafler DA. Regulatory T cells in autoimmune disease. Nat Immunol. 2018 Jul;19 (7):665–673. doi: 10.1038/s41590-018-0120-4

[21] Czarnowicki T, He HY, Wen HC, et al. Alopecia areata is characterized by expansion of circulating Th2/Tc2/Th22, within the skin-homing and systemic T-cell populations. Allergy. 2018 Mar;73(3):713–723. doi: 10.1111/all.13346

[22] Klarquist J, Denman CJ, Hernandez C, et al. Reduced skin homing by functional Treg in vitiligo. Pigment Cell & Melanoma Res. 2010 Apr;23(2):276–286. doi: 10.1111/j.1755-148X.2010.00688.x

[23] Kataoka H, Yasuda S, Fukaya S, et al. Decreased expression of Runx1 and lowered proportion of Foxp3 + CD25 + CD4 + regulatory T cells in systemic sclerosis. Mod Rheumatol. 2015 Jan;25(1):90–95. doi: 10.3109/14397595.2014.899736

[24] Radonjic-Hoesli S, Hofmeier KS, Micaletto S, et al. Urticaria and Angioedema: an update on classification and pathogenesis. Clinic Rev Allerg Immunol. 2018 Feb;54(1):88–101. doi: 10.1007/s12016-017-8628-1

[25] Olewicz-Gawlik A, Kowala-Piaskowska A. Self-reactive IgE and anti-IgE therapy in autoimmune diseases. Front Pharmacol. 2023;14:1112917. doi: 10.3389/fphar.2023.1112917

[26] Abdel-Gadir A, Schneider L, Casini A, et al. Oral immunotherapy with omalizumab reverses the Th2 cell-like programme of regulatory T cells and restores their function. Clin Exp Allergy. 2018 Jul;48 (7):825–836. doi: 10.1111/cea.13161

[27] Amat F, Tallon P, Foray AP, et al. Control of asthma by omalizumab: the role of CD 4 + Foxp3 + regulatory T cells. Clin Exp Allergy. 2016 Dec;46(12):1614–1616. doi: 10.1111/cea.12839

[28] Lopez-Abente J, Benito-Villalvilla C, Jaumont X, et al. Omalizumab restores the ability of human plasmacytoid dendritic cells to induce Foxp3 + tregs. Eur Respir J. 2021 Jan;57(1):2000751. doi: 10.1183/13993003.00751-2020

[29] Tai Y, Sakamoto K, Takano A, et al. Dysregulation of humoral immunity in Foxp3 conditional-knockout mice. Biochem Biophys Res Commun. 2019 Jun 11;513(4):787–793. doi: 10.1016/j.bbrc.2019.04.090

[30] Halabi-Tawil M, Ruemmele FM, Fraitag S, et al. Cutaneous manifestations of immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. Br J Dermatol. 2009 Mar;160(3):645–651. doi: 10.1111/j.1365-2133.2008.08835.x

[31] Nieves DS, Phipps RP, Pollock SJ, et al. Dermatologic and immunologic findings in the immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. Arch Dermatol. 2004 Apr;140(4):466–472. doi: 10.1001/archderm.140.4.466

[32] Lin W, Truong N, Grossman WJ, et al. Allergic dysregulation and hyperimmunoglobulinemia E in Foxp3 mutant mice. J Allergy Clin Immunol. 2005 Nov;116 (5):1106–1115. doi: 10.1016/j.jaci.2005.08.046

[33] Mercer F, Kozhaya L, Unutmaz D, et al. Expression and function of TNF and IL-1 receptors on human regulatory T cells. PLOS ONE. 2010 Jan 11;5(1): e8639. doi: 10.1371/journal.pone.0008639

[34] Goldmann O, Nwofor OV, Chen Q, et al. Mechanisms underlying immunosuppression by regulatory cells. Front Immunol. 2024;15:1328193. doi: 10.3389/fimmu.2024.1328193

[35] Ganesh BB, Bhattacharya P, Gopisetty A, et al. IL-1β promotes TGF-β1 and IL-2 dependent Foxp3 expression in regulatory T cells. PLOS ONE. 2011;6(7): e21949. doi: 10.1371/journal.pone.0021949

[36] Li L, Kim J, Boussiotis VA. IL-1β–mediated signals preferentially drive conversion of regulatory T cells but not conventional T cells into IL-17–producing cells. J Immunol. 2010 Oct 1;185(7):4148–4153. doi: 10.4049/jimmunol.1001536

[37] Sakaguchi S, Wing K, Onishi Y, et al. Regulatory T cells: how do they suppress immune responses? Int Immunol. 2009 Oct;21(10):1105–1111. doi: 10.1093/intimm/dxp095

[38] Lin TJ, Garduno R, Boudreau RT, et al. Pseudomonas aeruginosa activates human mast cells to induce neutrophil transendothelial migration via mast cell-derived IL-1α and β. J Immunol. 2002 Oct 15;169(8):4522–4530. doi: 10.4049/jimmunol.169.8.4522

[39] Guma M, Kashiwakura J, Crain B, et al. JNK1 controls mast cell degranulation and IL-1β production in inflammatory arthritis. Proc Natl Acad Sci USA. 2010 Dec 21;107(51):22122–22127. doi: 10.1073/pnas.1016401107

[40] Nakamura Y, Franchi L, Kambe N, et al. Critical role for mast cells in interleukin-1β-driven skin inflammation associated with an activating mutation in the Nlrp3 protein. Immunity. 2012 Jul 27;37(1):85–95. doi: 10.1016/j.immuni.2012.04.013

[41] Kim GY, Lee JW, Ryu HC, et al. Proinflammatory cytokine IL-1β stimulates IL-8 synthesis in mast cells via a leukotriene B4 receptor 2-linked pathway, contributing to angiogenesis. J Immunol. 2010 Apr 1;184(7):3946–3954. doi: 10.4049/jimmunol.0901735

[42] Venkateswarlu K. Analysis of the interaction between cytohesin 2 and IPCEF1. Methods Enzymol. 2005;404:252–266.

[43] Oh SJ, Santy LC. Differential effects of cytohesins 2 and 3 on β1 integrin recycling. J Biol Chem. 2010 May 7;285(19):14610–14616. doi: 10.1074/jbc.M109.043935

[44] Marks KE, Flaherty S, Patterson KM, et al. Toll-like receptor 2 induces pathogenicity in Th17 cells and reveals a role for IPCEF in regulating Th17 cell migration. Cell Rep. 2021 Jun 29;35(13):109303. doi: 10.1016/j.celrep.2021.109303

[45] Ferreira MAR, Vonk JM, Baurecht H, et al. Eleven loci with new reproducible genetic associations with allergic disease risk. J Allergy Clin Immunol. 2019 Feb;143 (2):691–699. doi: 10.1016/j.jaci.2018.03.012

[46] Ortiz RA, Barnes KC. Genetics of allergic diseases. Immunol Allergy Clin North Am. 2015 Feb;35 (1):19–44. doi: 10.1016/j.iac.2014.09.014

[47] Wang Z, Ji N, Chen Z, et al. MiR-1165-3p suppresses Th2 differentiation via targeting IL-13 and PPM1A in a mouse model of allergic airway inflammation. Allergy Asthma Immunol Res. 2020 Sep;12(5):859–876. doi: 10.4168/aair.2020.12.5.859

[48] Lin CE, Kaptein JS, Sheikh J. Differential expression of microRNAs and their possible roles in patients with chronic idiopathic urticaria and active hives. Allergy & Rhinol. 2017 Jun 1;8(2):67–80. doi: 10.2500/ar.2017.8.0199

[49] Jiang Y, Forno E, Han YY, et al. A genome-wide study of DNA methylation in white blood cells and asthma in Latino children and youth. Epigenetics. 2021 May;16 (5):577–585. doi: 10.1080/15592294.2020.1809872

[50] Lu LQ, Liao W. Screening and functional pathway analysis of genes associated with pediatric allergic asthma using a DNA microarray. Mol Med Rep. 2015 Jun;11(6):4197–4203. doi: 10.3892/mmr.2015.3277

[51] Hirota T, Takahashi A, Kubo M, et al. Genome-wide association study identifies eight new susceptibility loci for atopic dermatitis in the Japanese population. Nat Genet. 2012 Nov;44(11):1222–1226. doi: 10.1038/ng.2438

[52] Li Y, Qi X, Zhao D, et al. IL-3 expands pre-basophil and mast cell progenitors by upregulating the IL-3 receptor expression. Cell Immunol. 2022 Apr;374:104498. doi: 10.1016/j.cellimm.2022.104498

[53] Ferrer M, Luquin E, Kaplan AP. IL3 effect on basophils histamine release upon stimulation with chronic urticaria sera. Allergy. 2003 Aug;58(8):802–807. doi: 10.1034/j.1398-9995.2003.00195.x

[54] Us Altay D, Onder S, Etgu F, et al. A newly identified myokine: irisin, and its relationship with chronic spontaneous urticaria and inflammation. Arch Dermatol Res. 2023 Apr;315(3):437–442. doi: 10.1007/s00403-022-02378-4

[55] Zellweger F, Buschor P, Hobi G, et al. IL-3 but not monomeric IgE regulates FcεRI levels and cell survival in primary human basophils. Cell Death Dis. 2018 May 1;9(5):510. doi: 10.1038/s41419-018-0526-9